

Figure 1

VH G250

D V K L V E S G G L V K L G G S L K L

1 GAC GTG AAG CTC GTG GAG TCT GGG GGA GGC TTA GTG AAG CTT GGA GGG TCC CTG AAA CTC

S C A A S G F T F S N Y Y M S W V R Q T
61 TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT AAC TAT TAC ATG TCT TGG GTT CGC CAG ACT
H1

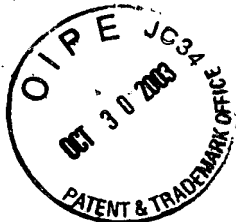
P E K R L E L V A A I N S D G G I T Y Y
121 CCA GAG AAG AGG CTG GAG TTG GTC GCA GCC ATT AAT AGT GAT GGT GGT ATC ACC TAC TAT
H2

L D T V K G R F T I S R D N A K N T L Y
181 CTA GAC ACT GTG AAG GGC CGA TTC ACC ATT TCA AGA GAC AAT GCC AAG AAC ACC CTG TAC

L Q M S S L K S E D T A L F Y C A R H R
241 CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC ACA GCC TTG TTT TAC TGT GCA AGA CAC CGC

S G Y F S M D Y W G Q G T S V T V S S
301 TCG GGC TAC TTT TCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA
H3

CDRs H1, H2, H3.
CDR definition according to Kabat scheme



VL G250

D I V M T Q S Q R F M S T T V G D R V S
1 GAC ATT GTG ATG ACC CAG TCT CAA AGA TTC ATG TCC ACA ACA GTA GGA GAC AGG GTC AGC

I T C K A S Q N V V S A V A W Y Q Q K P
61 ATC ACC TGC AAG GCC AGT CAG AAT GTG GTT TCT GCT GTT GCC TGG TAT CAA CAG AAA CCA

L1

G Q S P K L L I Y S A S N R Y T G V P D
121 GGA CAA TCT CCT AAA CTA CTG ATT TAC TCA GCA TCC AAT CGG TAC ACT GGA GTC CCT GAT

L2

R F T G S G S G T D F T L T I S N M Q S
181 CGC TTC ACA GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATT AGC AAT ATG CAG TCT

E D L A D F F C Q Q Y S N Y P W T F G G
241 GAA GAC CTG GCT GAT TTT TTC TGT CAA CAA TAT AGC AAC TAT CCG TGG ACG TTC GGT GGA

L3

G T K L E I K
301 GGC ACC AAG CTG GAA ATC AAA

CDRs L1, L2, L3

CDR definition according to Kabat scheme

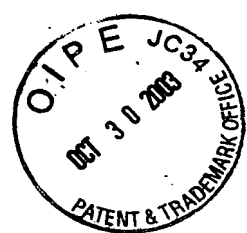


Figure 2

Primers used for PCR amplification of G250 VH and VL regions

5

Anchor and anchor poly C primers:

Anchor: 5'-GCA TGC GCG CGG CCG CGG AGG CC-3'

10

Anchor poly C: 5'-GCA TGC GCG CGG CCG CGG AGG CC(C)₁₂-3'

Constant primers:

15

VH-primers: 5'-CTC TAA GCT TGG CTC AAA CAC AGC
GAC CTC GGA TAC AGT TGG TGC AGC-3'

VL-primers: 5'-CTC TTC TAG AGA GTC TCT CAG CTG
GTA GGA TAC AGT TGG TGC AGC-3'



Figure 3A

08.05.2001

double strand sequencing

HC_cG250 (EcoRI-fragment in expression vector)

1 TCATGACATT AACCTATMAA AATAGGCGTA TCACGAGGCC CTTTCGTCTT CAAGAATCTT TCAGATACAA AGAATCTCTA AACCTGAGG ACATTCTATC 100
101 ACAATAAAGT AAAATTCAGA AAATCTGAA TGCCTCCATC ACAGAGATGA ATCTGCTATG AACAGCTCAT AGGTGTGAAG CTCTACAAA GCCATATTTAT 200
201 TGAAAAGCCA CATTGTGCC AGACTTTGGA AAGACTGAGC TCATATCCTG AAATACAGTT ATGTGTGGTT CTATCTTANTT ACACATTTAC ACTAAGGAAA 300
301 CATGGCAGTA TGGGAATGAA GCTTGTCTG TACACATTA CAGAGGGAAA CTTAAACAAG TATGGTGAAT CCTTACCMA AAGTAAAAAA AAAAAAAA 400
401 AAGAAAGAA AAGAAAAAA AAGTGAACCT ACAATATGTT TCAAAATGCTG TAACTGAANT CTGGTTTTTT GATGCCTTAT ATCTGTTATC ATCAGTGAAT 500
501 TCAGATTAG TCCAACCTCA GAGCATGGTA TAGCAGGAAG ACATGCNAAT AGGTCTTCTC TGTGCCCATG AAAAAACACCT CGGCCCTGAC CCTGCAGCTC 600
601 TGACAGAGGA GGCCTGTCTT GGATTCGATT CCCAGTTCTT CACATTCAGT GATCAGCACT GAACACAGAC CCTCACCAT GAACCTCGG CTCAGANTGA 700
701 TTTTCTCTGT CCTGTGTTTA AAGGTATCT TATTGATAT AGAGACATC TGCTGTATGC ACAGAGGTGC AGAAAAATG TTTTGTGTTT TTTTGTGTA 800
801 CAATGCTCCA AACAGTATTC TTTCTTTGCA GGTGTCTCTT GTGACGTGAA GCTCGTGGAG TCTGGGGGAG GCTTGTGTA GCTTGTGAGG TCCCTGAAAAC 900
901 TCTCTGTGTC AGCCTCTGGA TTCACTTTCA GTAACTATTA CATGTCTTGG GTTCGCCAGA CTCCAGAGAA GAGGCTGGAG TTGTCGCAG CCATTAATAG 1000
1001 TGATGGTGGT ATCACCCTACT ATCTAGACAC TGTGAAGGC CGATTACCA TTTCAGAGA CAATGCCAAG AACACCTCTT ACCTGCAAT GAGCAGTCTG 1100
1101 AAGCTGAGG ACACAGCCCT GTTTTACTGT GCAGACACC GCTCGGGCTA CTTTCTTATG GACTACTGG GTCAAGGAAC CTCAGTCACC GTCTCCTCAG 1200
1201 GTAAGAAATGG CCTCTCCAGG TCTTTTTTTT AATCTTTGTA ATGGAGTTTT CTGAACATG CAGACTAATC TTGGATATTT GTCCCTGAGG TAGCCGGCTG 1300
1301 AGAGAAATG GGAATTAAC TGCTCGGGA TCTCAGAGCC TTTAGGACAG ATTATCTCCA CATCTTTGAA AACTGAGAT TCTGTGTGAT GGTGTTGGTG 1400
1401 GAGTCCCTGG ATGATGGGAT AGGACCTTTG GAGGCTCANT TGAGGGAGAT GCTAAACAA TCCTATGGCT GGAGGAGAG TTGGGGCTGT AGTTGGAGAT 1500
1501 TTTTCAGTTT TAGAATAAAA GCTTTAGCTG CGGGAATCC TTCAGGACCA CCTCTGTGAC AGCATTTATA CAGTATCCGA TGCATAGGGA CAAAGAGTGG 1600
1601 ACTGGGGCAC TTTCCTTGA TTTGTGGGGA ATGTTCCACA CTAGTTTCTG TGAACCTCA TTTGTGGAG GGAGAGCTGT CTTAGTGCCT GAGTCAAGGG 1700
1701 AGRAGGGCAT CTAGCCTCGG TCTCAAAAG GTAGTTGCTG TCCAGAGAGG TCTGGTGGAG CCTGCAAAAG TCCAGCTTTC AAAGGAACAC AGAAGTATGT 1800
1801 GTATGGAATA ATAGAAGATG TTGCTTTTAC TCTTAAGTTG GTTCATAGGA AAAATAGTTA AACTGTGAG TTTAAAAATG GAGAGGGTTT TCAAGTACTC 1900
1901 ATTTTCTTAC ATGTCCAAA TTTCTGTCAA TCAATTTGAG GTCTGTGTTG TGTAGAACTG ACATTTACTTA AAGTTTAACC GAGGAATGG AGTGAGGCTC 2000
2001 TCTCATACCC, TATTACAGAC TGACTTTTAA CAAATATTA TTAAGTTTAA AATTTTTTA AATGATTTGA GCAATGTTGA GTTGGAGTCA AGATGGCCGA 2100



10.05.2001

double strand sequencing

LC_cG250 (HindIII-fragment in expression vector)

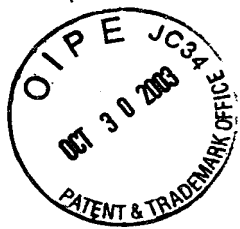
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4601 GAAGTCAAGT TCAGCTCCTA AATGGATGT GGGAGCAAAAC TTTGAGATA AACTGAATGA CCCAGAGGAT GAACAGTGC AGATCAAAAGA GGGGCTTGA 4700
4701 GCTCTGAGAA CAGAAAGGAGA GTCATTCGTG TTGAGTTTCC ACAAACTAG TCTTGAGTTT TGCAATAAAA GTGGGATAGC AGAGTTGAGT GAGCCATAGG 4800
4801 CTGAGTCTC TCTTTTGTCT CCTAAGTTT TATGACTACA AAATCAGTA GTATGTCCCTG AAATAATCAT TAACTGTTT GAAAGTATGA CTGCTTGCCA 4900
4901 TGTAGATACC ATGGCTTGCT GAATAATCAG AAGAGGTGTG ACTCTTATTC TAAAAATTTGT CACAAAATGT CAAAATGAGA GACTCTGTAG GAACGAGTCC 5000
5001 TTGACAGACA GCTCAAGGGG TTTTTCCT TTGTCCTCAT TCTACATGAA AGTAAATTTG AAATGATCTT TTTTATTATA ATAGTAGAAA TACAGTTGGG 5100
5101 TTTGAACTAT ATGTTTAAAT GGCCACGGT TTGTAAGACA TTTGGCCCTT TGTATTTCCCA GTTATTTACTC GCTTGTAAAT TTTATATCGCC AGCAATGGAC 5200
5201 TGAACGGTC CGCAACCTCT TCTTTACAAC TGGGTGACCT CGCGGCTGTG CCAGCCATTT GCGGTTCCACC TTGCCGCTAA GGGCCGTGTG AACCCCGAG 5300
5301 GTAGCATCCC TTGCTCCGG TGGACCACTT TCCTGAGGCA CAGTGATAGG AACAGAGCCA CTAATCTGAA GAGAACAGAG ATGTGACAGA CTACACTAAT 5400
5401 GTTAGAAAAA CAAGGAAGG GTGACTTATT GGAGATTTCA GAAATAAAT GCATTTATTA TTATATTTCC TTATTTTAAAT TTTCTATTAG GGAATTAGAA 5500
5501 AGGCATAAA CTGCTTTATC CAGTGTATA TTAAAGCTT TTTTTCCTC AGTGCTA 5557
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Figure 3B:

LC_cG250 (HindIII-fragment in expression vector) double strand sequencing 10.05.2001

1	AATTCGAAGC	TTTGTATCTT	CAGATCCAGG	AAAGCCACCA	CCAAATACAA	ACAGATACAT	GCTGAACCA	ACTTCTGTTT	TTATGTCAA	TGCACAGCGG	100
101	GCATCTGACA	CTGCCCTGCAT	GAAGTCTCA	GGTCAANTACT	TCCACTACAC	ACATGGAAGC	TGACACCAAT	GAGTCAATA	TTTAGTCTTT	TTGCCCATTT	200
201	CAGGAGGAGA	CTGCTGGTTT	TGAGTGTGGC	ACCAGACTTA	ACACCAAGTC	GACAAACTGC	TTTGGAAATCA	TCTGTGACAA	TCCACAAAAA	CNACTTTGTC	300
301	TTACAANTGT	CTCTGACGAC	ATTCAATCAAT	TCAATTTCACT	GTCAAAAAGTC	ATCATCTGGA	CTCCATTTACT	GGCAGCATAC	TTGAATTTGAG	ACACTTTGTT	400
401	ACAAAAAATGT	GCATAGGTAA	TCCTCTCTGG	AGGAACCCAGA	AGCCCCCGTT	CCAACTGTAT	TTTCACTCTTG	CTTGACACAGT	CAANTCCTGT	ACCAATAGCA	500
501	GCTAGGGTGT	TAACTATGGC	TCTGTGTGTC	TTACACTTGA	CTGCACAAAA	AGGAATAACA	TTTCGGAAGAG	CTTTTAGCCA	CCTCAGATGC	TTCTTTTAGAA	600
601	TGCTCTCTGAG	GTCCGGAACC	TAGAAAGAAG	AGACTTCAAT	TATTAATTTTG	TGTTTCAGAA	GTCTTTAGCA	CTAAAGCCAC	CATCTATGAT	ACAGCAGTCA	700
701	AACTCTTCTCT	TAGTATAGCT	GCTCATCGTT	CTCCATGTGC	CTACAGAAAA	CCTAGACATG	GAATTAATTT	ATTGCCAGCC	CCTTACAAGG	TCAACTTATC	800
801	CAAGAACTGT	GAATGCAGAC	TCCTTGAAT	GTTCGAAACA	CTCACAGCAC	AGGTCAAGA	CTGGCTGGAC	ACATGGAGAC	ACTGAATCCT	GAAGAGCACT	900
901	TAGCTGTCTG	TTGCTTCATC	ATGCTACTG	ACCTGAGGTG	GCACCAAGCT	GCCTACTGAG	GGAGGACITGT	GGCGGTGCT	GCAGGAACCTG	ACAATTTCTC	1000
1001	ACAATTTCTCT	TACTGCCCCA	CTCATAACTC	TTCTCTTCTC	CATCTTCTTC	TTTCTTTTCT	CTTCCCTCCT	TTTTCCCTTT	CACACTTTTT	TTCTTTTCTT	1100
1101	CTTTTCCACT	TCCCTTTTCT	TTCTTTCTTT	GCTGTGTGCTG	TTGTAAAGGA	TTTATTTGTTT	CCCTGTGATT	GAACCAAGG	TAGTTGTACT	ATTATTTCTG	1200
1201	TAAAACTCAT	CTGTGTGATT	TCTATTAAT	AATTAATTTT	GTTTACACTC	CATATTTTAT	TCAACCCCTC	CATCTCCTA	CTGGTCTACA	TACCATACCT	1300
1301	CTTTCCACCA	CCCCGTGCTC	CACATGGATG	CTGCCACCTC	CCATGCCACC	TGACCTCTCA	TCTCCCTAGG	GCATCTAGTC	TCTTGAGGCT	TAGATGCATC	1400
1401	ATTTCTGAGT	GAACACAGAT	CCAACAATCC	TCTGTATAT	GTGTGTGTGT	GGCCTCATAG	CAGCTGGTGT	ATGCTGCCCTG	TTTGTGTGATC	CAGTGTTTGA	1500
1501	GAGGTCTCGC	GGGTTTCAGAT	TAAATGAGAT	TGTTGGACCT	CCTCAGCGTC	TTTTCAGTCTT	TCCCTGATTC	AACAACAGGG	TTTCAATTTT	CTGTTCATTTG	1600
1601	GTGGGTGCA	AATATCTGCA	TCTGACTCAG	CTGCTTATTG	GGTCTTCTGG	AGTGCAGTCA	TGCTAGGTCC	GTTCCTATGA	GTGCTCCATA	GCCTCAGTGA	1700
1701	TAGTGTGAGG	CGTTGGGACT	GCCCCCTTGAC	CTGGATTTCTA	TTTTGGACCT	GTGCTGGGAC	CTTCTTTTCC	TCAGGCTCCC	CTCCATCTGT	ATCCCTGTAA	1800
1801	TTCTTTTACA	CAGGAACAAA	TATGGGTACAG	AGTTGTGAGT	GTGGAATGGC	ACCCCTTCTC	CTCATTTAAT	GGCCTGTCTT	CCTGGTGGAA	GTGGGCTCTA	1900
1901	TAACTTCCA	CTCCCTACTG	TTGGGCAATTT	CATCCCTTTG	AGTCTTGAGA	GTCTCTCACC	TCCCAGGTCT	CTGGTGCAAT	CTGGAGGGTC	CTCCCACTT	2000
2001	CTTACCTCCC	CAGGTTGCCT	GTTCACAGAC	TTCTGTCTGGC	CCCGATGCT	TCAGTCTCTT	TCCCTCACCC	AATATCTGAT	TTGGATGGAA	GCCTGTCTATG	2100



HC_cG250 (EcoRI-fragment in expression vector) double strand sequencing 08.05.2001

2101 TCAGAACCCAG AACACCTGCA GCAGCTGGCA GGAAGCAGGT CATGTGGCAA GGCTATTTGG GGAAGGGAAA ATAAACCAC TAGGTAACT TGTAGCTGTG 2200

2201 GTTTGAAGAA GTGGTTTTGA AACACTCTGT CCAGCCCCAC CAAACCGAAA GTCCAGGCTG AGCAAAACAC CACCTGGGTA ATTTGCATTT CTAAATAAAG 2300

2301 TTGAGGATTC AGCCGAAACT GGAGAGGTCC TCCTTTAACT TATGAGTTC AACCTTTTAA TTTTAGCTTG AGTAGTCTA GTTCCCCCA ACTTAAGTTT 2400

2401 ATCGACTTCT AAATGTATT TAGAATTCAT T 2431



LC_cG250 (HindIII-fragment in expression vector) double strand sequencing 10.05.2001

2101 AGAACATCTA TATACITG TGTTTCAGAGC TTAAATATGG TCCTTGAGCT TCTATTTTGA GTTCCTTTCC AGTGATTACT TGCITGCTTTT GGTAGTACTT 2200
2201 TTGACTGTTT ATTAAACCTG GATACTCTCA TACAGCTGTG TAATTTACTT CCTTATTTGA TGACTGCTTT GCATAGATCC CTAGAGGCCA GCCCAGCTGC 2300
2301 CCATGATTTA TAAACCAGGT CTTTGCACTG AGATCTGAAA TACATCAGAA CAGCATGGGC TTCAAGATGG AGTTTCATAC TCAGGTCTTT GTATTGCTGT 2400
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2601 GAGACATGTT GATGACCCAG TCTCAAGAT TCAATGTCAC AACAGTAGGA GACAGGGTCA GCATCACCCTG CAAGGCCAGT CAGATGTGG TTCTTGCTGT 2700
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2901 GAGGCACCAA GCTGGAAATC AAGGTAAAT AGAATCCAAA CTCTCTTTCT TCCGTTGTCT ATGCTGTGG CTTCTATGTC TAAATATGAT GTAGATATTT 3000
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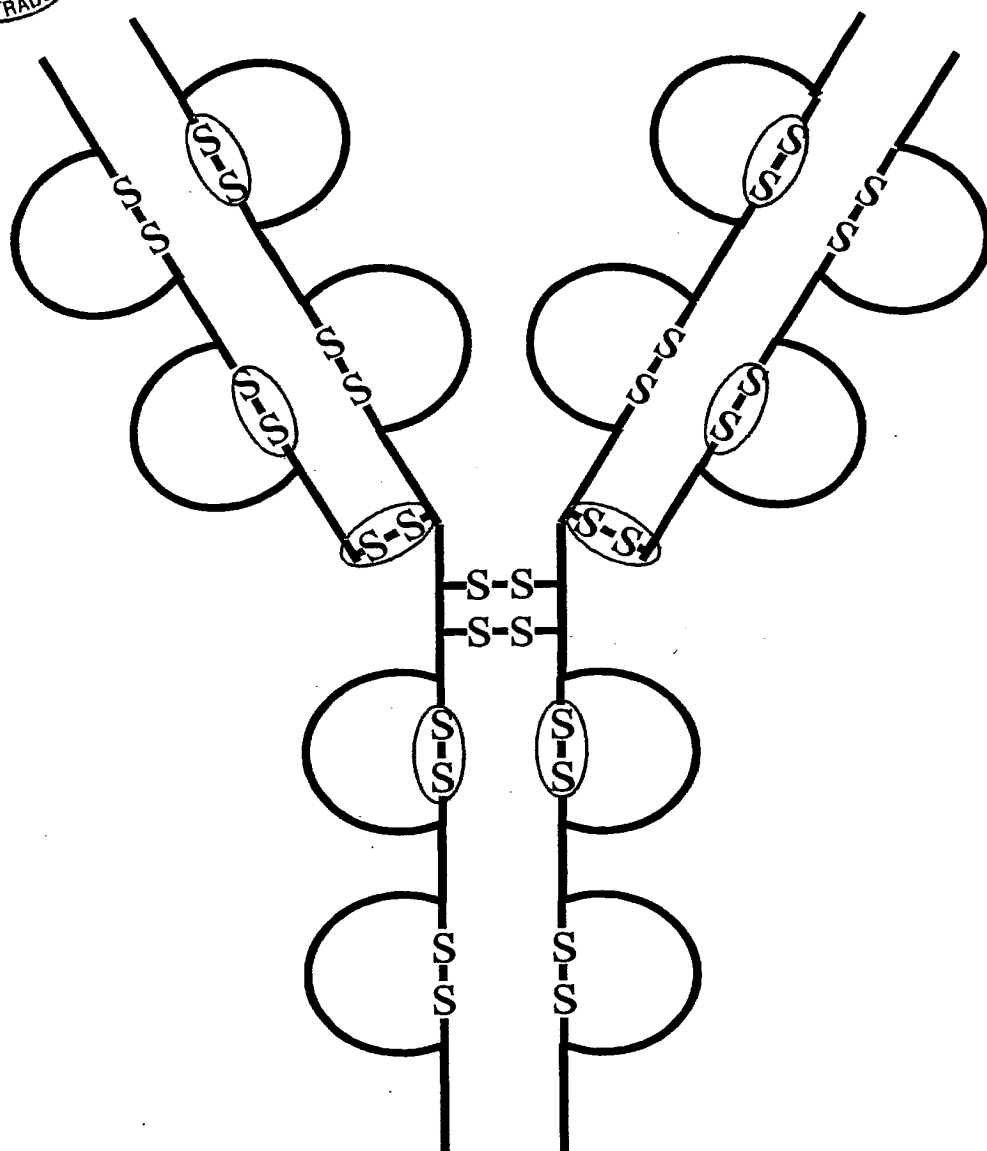


Figure 4

Schematic figure of an antibody. Disulfide bonds detected by mass spectrometry after enzymatic digest in WX-G250 are labeled by ovals.

Figure 5

Characterization of WX-G250 according to MALDI-PMF (Trypsin, LysC, AspN, GluC, and BrCN) in reflector and linear mod



Light Chain:

DIVMTQSQR F MSTTVGDRVS IT C KASQNVV SAVAWYQQKP

GQSPKLLIYS ASNRYTGVPD RFTGSGSGTD FTLTISNMQS

EDLADFE C QQ YSNYPWTEGG GTKLEIKRTV AAPSVFIFPP

SDEQLKSGTA SVV C LLNNFY PREAKVQWKV DNALQSGNSQ

ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYA C EVTHQG

LSSPVTKSFN RGE C

Heavy Chain:

DVKLVESGGG LVKLGGSLKL S C AASGFTES NYMSWVRQT

PEKRLELVAA INSDGGITYY LDTVKGRTI SRDNAKNTLY

LQMSSLKSED TALFY C ARHR SGYFSMDYWG QGTSVTVSSA

STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW

NSGALTSGVH TFAVLQSSG LYSLSVVTV PSSSLGTQTY

I C NVNHKPSN TKVDKKVEPK SCDKTH C PP C PAPELLGGP

SVLEFPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY

VDGVEVHNAK TKPREEQYNS C TYRVVSVLTV LHQDWLNGKE

YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL

TKNQVSLT C L VKGFYPSDIA VEWESNGQPE NNYKTTPPVL

DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ

KSLSLSPG C

----- Reflector mode
----- Additional information
----- from linear mode spectra

C Detected bridged cysteins (reflector mode)
C Detected bridged cysteins (linear mode)
C Cystein not determined as bridged cysteins
C Heavy chain partially lacks C-terminal lysine



Confirmed glycosylation site



Confirmed deamidation sites



Light chain:

DIVMTQSQRF MSTTVGDRVS ITCKASQNVV SAVAWYQOKP GQSPKLLIYS
ASNRYTGVPD RFTGSGSGTD FTLTISNMQS EDLADFFCQQ YSNYPWTFGG
GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEC

Heavy chain:

DVKLVESGGG LVKLGGSLKL SCAASGFTFS NYMSWVRQT PEKRLELVAA
INSDGGITYY LDTVKGRTI SRDNAKNTLY LQMSSLKSED TALFYCARHR
SGYFSMDYWG QGTSVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFAVLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

Figure 6: LC-MS and LC-MS/MS of tryptic digest of cG250